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RESEARCH ARTICLE

KERATINASE ACTIVITY OF DERMATOPHYTES AND YEAST SPECIES FOR POULTRY WASTE AND WASTE WATER TREATMENT

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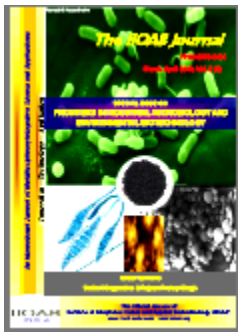
ABSTRACT



Dermatophyte and yeast species have been screened for their degradative ability towards various keratin substrates. Application of soluble preparation of keratin (KS) of chicken feathers enables a preliminary evaluation of the growth of the fungi and screening of fungal isolates that possesed keratinolytic activity and keratinase enzyme. Five dermatophytes i.e. Trichophyton rubrum, Trichophyton mentagrophytes, Microsporum gypseum, Microsporum canis and Chysosporium tropicum and two yeasts i.e. Candida albicans and Malassezia furfur were used in this study for keratinase activity. Out of five dermatophytes species and two yeast species studied, all tested fungal species showed a keratinase activity except one species i.e. M.canis. From findings, the results provide a scientific validation for the use of these microorganisms (dermatophytes and yeast species) for keratinase enzyme activity for the treatment of poultry waste and sewage waste water treatment.

Key words: ***keratinase; enzyme; keratin; sewage; dermatophytes; yeasts***

[I] INTRODUCTION

Feather is composed of over 90% protein, the main component being keratin, a fibrous and insoluble protein highly cross-linked with disulphide and other bonds. The feather can be hydrolysed by keratinase which is a proteolytic enzyme specific to keratins [1]. The keratinase enzyme is a potential enzyme for removing hair and feather in the poultry industry [2]. This enzyme has been produced by fungi, including the species of Aspergillus, Onygena, Absidia and Rhizomucor. Some species of dermatophytes, including *Trichophyton mentagrophytes*, *T. rubrum*, *T.gallinae*, *Microsporum canis* and *M.gypseum* [3]. Keratinophilic fungi was also screened for their degradative ability towards various keratin substrates like hair, nails, feathers etc [4]. Suntornsuk and Suntornsuk reported growth and efficient utilization of feather by Bacillus sp. FK 46 with release of 0.9 unit/ml of keratinase [5]. Doratomyces microsporus also produce keratinase enzyme and degrade skin epidermis in vitro under different experimental conditions [6]. Hydrolysis of feathers by microorganisms possessing keratinolytic activity represents an attractive alternatives method for improving the nutritional value of feather meal, compared to currently used physiochemical methods [7]. Most of the fungi exhibited variable efficiency in producing extracellular keratinase when grown in plates with chicken feathers as the sole carbon and nitrogen source [8]. In the present study, keratinase activity of dermatophytes and yeast species were investigated with the aim to use the keratinase enzyme for various biotechnological applications like removal of hair and feathers in leather and poultry industries, in sewage system for cleaning obstructions during waste water treatment and digestion of abundant waste generated from poultry processing industries.

[II] MATERIALS AND METHODS

Keratinase activity or degradation of keratin substrate (chicken feathers) by keratinolytic fungi was studied by agar plate method [3].

2.1. Keratin media

The keratinolytic properties of the fungi were examined on a solid mineral medium according to Wawrzkiewicz et al. (1991) [3]. The media

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was supplemented with the keratin substrate as the sole sources of carbon and nitrogen. Chicken feathers or a soluble preparation of keratin protein of chicken feathers described as KS (Soluble keratin) [3].

2.2. Preparation of keratin substrate

Preparation of soluble keratin protein is constituted from white chicken feathers where 10 gms of native chicken feathers where 10 gm native chicken feathers is dissolved with 500ml of DMSO (Dimethylsulfoxide) by heating at 100C temperature for 2 hours. Solution is precipitated for soluble protein (keratin) by using 2 volume of cold acetone for 1 volume of protein and then caseous precipitate of keratin protein was suspended in 0.1 M of phosphate buffer. Afterwards, soluble keratin protein was added in the solid media at a concentration of 0.06%. Solid media were inoculated with micropipette, injecting 1ml of standard suspension into centre of the petriplate and petriplates were incubated at 37ºC at an optimum pH 7.4 for keratinase activity. Control plates were prepared containing above medium without keratin substrate. Formation of precipitate zones around the colonies indicate keratinase enzyme production and zone was examined and measured.

[III] RESULTS

Keratinase activity of dermatophytes and yeasts were studied under in vitro laboratory conditions on the solid mineral medium incorporated with soluble preparation of keratin protein (KS). Keratin substrate used for in vitro degradation was chicken feathers. Five dermatophytes i.e. *T. rubrum*, T. mentagrophytes, M. gypseum, M. canis and C. tropicum and two yeasts i.e. C. albicans and M. furfur were used in our study for keratinase activity. All fungal isolates used in our study, grew on the solid mineral medium containing 0.06% of keratin protein (KS) with 14 days of incubation. Out of five dermatophytes species and two yeast species studied, all tested fungal species showed a keratinase activity except one species i.e. *M. canis* **[Table– 1 and Figure–1]**.

Table: 1. Keratinase activity of fungal isolates on solid mineral medium

Fungal species	Incubation		
	3	10	14
C. albicans	12*/40**	12*/40**	12*/40**
M. furfur	10*/40**	13*/47**	13*/47**
T. rubrum	-	20*/0**	20*/45**
T. mentagrophyts	20*/0**	20*/26**	22*/49**
M. gypseum	-	20*/34**	47*/65**
M. canis	-	-	20/0
C. tropicum	10*/20**	15*/25**	40*/60**

*Indicates fungal colony diameter; ** Indicates diameter of the zone of keratin solubilization; - Indicates no growth of the fungus; All numbers represent the average of triplicates; Control showed growth of selected test organisms but not cleared zone around fungal colonies

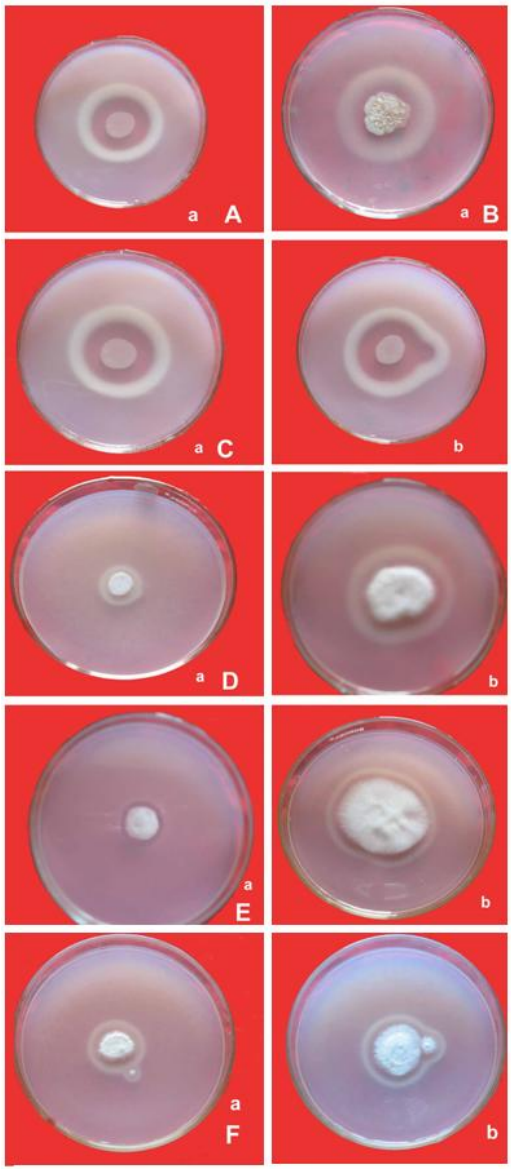

Fig: 1.keratinase activity of fungi. A. *C. albicans:* (a) at third, tenth and fourteenth day of incubation. **B.** *T. rubrum*: (a) at fourteenth day of incubation. **C.** *M. furfur*: (a) at third day of incubation, (b) at tenth and fourteenth day of incubation. **D.** *T. mentagrophytes*: (a) at tenth day of incubation, (b) at fourteenth day of incubation. **E.** *M. gypseum*: (a) at tenth day of incubation, (b) at fourteenth day of incubation. **F.** *C. tropicum*: (a) at third day of incubation, (b) at tenth and fourteenth day of incubation.

It was found that, the application of soluble preparation of keratin (KS) of chicken feathers enables a preliminary evaluation of the growth of the fungi and screening of fungal isolates that possesed keratinolytic activity and keratinase enzyme. C. albicans also showed a good keratinase activity. On third day of incubation colonies of *C. albicans* (12 mm in diameter) were surrounded by broad zone of (40 mm) of

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degraded keratin. Within an extended period of incubation (10 and 14 days), no change in the zone diameter was observed. M. furfur also showed good keratinase activity when compared with C. albicans. Colonies (about 10 mm in diameter) was surrounded by broad zone of (40 mm) of degraded keratin within three days of incubation. At extended incubation period (10 and 14 days) clear zone of 47 mm was found. *T. rubrum* showed keratinase activity on a solid mineral medium within incubation of 14 days. No growth of *T. rubrum* was observed on medium at third day of incubation. *T. rubrum* showed 20 mm diameter of fungal colony and no degradation on tenth day but on fourteenth day of incubation, *T. rubrum* colony (20 mm in diameter) was surrounded by a broad zone (45 mm in diameter) of degraded keratin, thus indicating an active secretion of keratin decomposing enzymes to the medium. T. mentagrophytes showed colony diameter of 20 mm at third day of incubation but clear zone of keratin degradation was not reported within 3 days but after on tenth day of incubation, colonies of (20 mm in diameter) surrounded by clear zone of (26 mm) diameter of degraded keratin was observed and on fourteenth day of incubation large broad zone (about 49mm in diameter) of keratin degradation was found. Formation of clear zone around fungal colonies was reported on both tenth and fourteenth days of incubation. Zone of keratin degradation of fourteenth day of incubation was broader and larger than tenth day of incubation period. Growth of M. gypseum was not observed on third day of incubation. At tenth day of incubation, both growth and clear zone of degradation was observed. Colony diameter of 20 mm surrounded by narrow clear zone of 34 mm was found and within fourteenth day of incubation, large broad zone of 65 mm was reported when compared with tenth day of incubation (34 mm in diameter). M. canis showed no growth on solid mineral medium at third day and tenth day of incubation. On fourteenth day of incubation, growth of 20 mm in diameter was reported but clear zone of keratin degradation was not observed around fungal colony. Thus indicating an absence of active secretion of keratin decomposing enzymes into the medium. The excellent keratinolytic activity was observed in C. tropicum. Within 3 days, this fungi grew on solid medium and also showed clear zone of 20 mm. Further increase in incubation period of 10 days, showed a higher zone of degradation (25 mm in diameter) than third day of incubation. On fourteen days of incubation, highest zone of keratin degradation of about 60 mm in diameter was reported. In the present study, increased keratinase enzyme activity for C.albicans, M.furfur, T.rubrum, T.mentagrophytes, M.gypseum and C.tropicum was found at optimum temperature i.e. 37±2°C and pH 7.4.

[IV] DISCUSSION

In this study, degradation of keratin substrate (chicken feathers) by dermatophytes and yeasts species were studied. It was found that, M. gypseum (65 mm in diameter) showed highest keratinase activity followed by C. tropicum (60 mm), T. mentagrophytes (49 mm), M.furfur (47 mm), *T. rubrum* (45 mm) and C. albicans (40 mm). In our study, all the tested fungal isolates showed a diameter of clear zone of keratin degradation within range of 40-65 mm and possessed good keratinase activity except one species, M. canis which showed growth on solid mineral medium but clear zone of keratin degradation was not found by further increase of incubation period from 14 days to 21 days. This reflected the fact that keratin is the main substrate for dermatophytes and therefore these fungi are called keratinophilic.

The examined species of dermatophytes and non dermatophytes (yeasts) were both keratinase procedures and they are apparently capable of damaging the keratinized structure of the skin as previously reported [3]. The high keratinase activity of *M. gypseum* in comparison with other related fungi explain their ability to invade chicken feathers and keratin degradation. This was in agreement with work of earlier workers [9,10] which revealed that *M.gypseum* showed highest keratinase activity among examined dermatophytes and non-dermatophytes.

The present experiments indicate a possibility of appearing modified keratin of chicken feathers / KS (Soluble keratin) as a very useful model for a preliminary estimation of keratinolytic activity of dermatophytes. This keratin, introduced as a source of carbon and nitrogen to the mineral agar medium, allows a quick selection of active strains. Native keratin contained in hairs or feathers did not constitute such a universal source of C and N for dermatophytes as the preparation of KS employed in our experiments. The few strains degrading keratin of guinea pig hair included strains of T.verrucosum and T. mentagrophytes of a wide infections spectrum [3]. In our studies, T. mentagrophytes also produced high keratinase activity (49 mm).

These results are also similar to Muhsin et al. [10] which revealed that the three tested varieties of T. mentagrophytes showed high keratinase activity. Sharma (2009) also reported that maximum keratinase (2.57± 0.028 unit/ml) was released from T.mentagrophytes when 35ºC temperature was provided [11]. In case of M. canis, keratinase activity was not detected. These result are in agreement with Wawrzkiewiez et al. [3] where M. canis was found negative for keratinase activity but disagree with the results of Muhsin et al [10] who reported (15 mm) zone of precipitation around fungal colony of *M. canis* [4]. Chrysosporium tropicum was also found in this study superior for keratinase production, forming zone of 60 mm after 14 days of incubation at 37°C. These results was in agreement with El-Naghy et al. (1998) [12] who reported that the Chrysosporium georgiae possessed high keratinase activity and completely degraded the added keratin after 9 days of incubation.

Moreira et al. (2007) [13] investigated, degradation of keratinous materials by the plant pathogenic fungus Myrothecium verrucaria using poultry feathers as the only substrate. According to da Gippo et al. (2009) [14], the association of two residues poultry feather powder (PFP) plus cassava bagasse could be an excellent option as a cheap culture

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medium for the production of keratinase in submerged and solid state cultures. New feather degrading filamentous fungi was studied by Rodrigues Marcondes et al. (2008) [15]. The study found that the highest keratinolytic activity were produced by Alternaria tenuissima after 4-6 days of cultivation in submerged conditions followed by *Acremonium hyalinulum, Curvularia branchyspora, Beauveria bassiana*. The results of this work contribute to showed that keratinolytic activity was relatively widespread among common filamentous fungi and may have an important role in feather decomposition. According to Gupta and Ramnani (2006), microbial keratinases have become biotechnologically important since they target the hydrolysis of highly rigid, strongly cross-linked structural polypeptide "keratin" recalcitrant to the commonly known proteolytic enzymes trypsin, pepsin and papain [16]. These enzyme are largely produced in the presence of keratinous substances in the form of hair, feather, wool, nail, horn etc. during their degradation. Raju et al. (2007) [17], in their studies, clearly indicates the presence of enzyme keratinases in the dermatophyte Microsporum gypseum and found that maximum biomass and keratinase activity was observed at pH 8 and at 35°C. Muhsin et al. (1997) [10] also found in their studies that *T. rubrum* and C. albicans also showed good keratinase activity which was coincides with the results of our present work. *T. rubrum* and C. albicans produced clear zone of 45 mm and 40 mm in our studies. Peyton and Weary (1968) found that M. furfur capable of degrading keratin. work agree with their studies. M.furfur showed positive keratinase activity but disagree with Muhsin et al. who reported negative keratinase activity in M. furfur. Difference in the properties of keratinases in particular strains of dermatophytes have been noted by Takiuchi et al[18].

[V] CONCLUSION

From the results, it is evident that dermatophytes and yeast species were capable of producing keratinase enzyme. Finally this study suggests that microorganisms (dermatophytes and yeast species) can be used to degrade keratin instead of using physiochemical methods for the removal of wastes of poultry industries and for cleaning obstructions in sewage waste water treatment.

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REFERENCES

[1] Friedrich J, Gradisar H, Mandin D, Chaumont JP. [1999] Screening fungi for synthesis of keratinolytic enzymes. *Lett Appl Microbiol* 28: 127−130.
[2] Takami H, Satoshi N, Aono R, Horikoshi K. [1992] Degradation of human hair by a thermostable alkaline protease from alkaliphilic Bacillus sp. no. AH-101. *Biosci Biotechnol Biochem* 56: 1667−1669.
[3] Wawrzkiewicz K, Wolski T, Lobarzewski J. [1991] Screening the keratinolytic activity of dermatophytes in vitro. *Mycopathologia* 114: 1−8.
[4] Muhsin TM, Hadi RB. [2002]. Degradation of keratin substrate by fungi isolated from sewage sludge. *J Mycopathol* 154(4): 185−189.
[5] Suntornsuk W, Suntornsuk L. [2003] Feather degradation by Bacillus sp. FK 46 in submerged cultivation. *Biores Technol* 86(3): 239−243.
[6] Gradisar H, Vricel M, Pogainik A, Friedrich J. [2004]. In vitro degradation by of porcine skin epidermis by a fungal keratinase of Doratomyces microsporus. *J Mycol* 34: 101−105.
[7] Bertsch A, Coello N. [2005] A biotechnological process for treatment and recycling poultry feathers as a feed ingredient. *Biores Technol* 96: 1703−1708.
[8] Anbu P, et al. [2006] Secretion of keratinolytic enzymes and keratinolysis by Scopulariopsis brevicaulis and Trichophyton mentagrophytes : regression analysis. *Can J Microbiol* 52(11): 1060−1069.
[9] Takiuchi I, Sei Y, Takagi M, Negi M. [1984]. Partial characterization of the extracellular keratinase from Microsporum canis. Sabouraudia. *J Med Vet Mycol* 22: 219−224.
[10] Muhsin TM, Aubaid AH, Al−Duboon A.H. [1997]. Extracellular enzyme activities of dermatophytes and yeast isolates on solid media. *Mycosis* 40: 465−469.
[11] Sharma A, Chandra S, Sharma M. [2010]. Prevalence of keratinophilic fungi in semi−arid region with particular reference to soil pH. *Asian J Exp Sci* 24: 59−63.
[12] El−Naghy HA, et al. [1998] Degradation of chicken feathers by Chrysosporum georgiae. *Mycopathologia* 143(2): 77−84.
[13] Moreira FG, et al. [2007] Degradation of keratinous materials by the plant pathogenic fungus Myrothecium verrucaria. *Mycopathologia* 163(3): 153−160.
[14] da Gippo NM, et al. [2009] Influence of the carbon and nitrogen sources on keratinose production by Myrothocium verrucaria in submerged and solid state cultures. *J Ind Microbiol Biotechnol* 36(5): 705−711.
[15] Rodrigues Marcondes N, Ledesma Taira C, Cirena Vandresen VD, et al. [2008] New feather degrading filamentous fungi. *Microb Ecol* 56(1): 13−17.
[16] Gupta R, Ramnani P. [2006] Microbial keratinases and their prospective applications: an overview. *Appl Microbiol Biotechnol* 70(1): 21−33.
[17] Raju KC, Neogi U, Saumya R, Goud, NR. [2007]. Studies on extracellular enzyme keratinase from dermatophyte Microsporum gypseum. Int. *J Biol Chem* 1: 174−178.
[18] Peyton E, Weary MD. [1968] Pityrosporum ovale. *Arch Dermatol* 98(4): 408−422.